CLAIMS

1. A polynucleotide separation method comprising the steps of:

immobilizing each of single stranded-oligonucleotide probes each having a specific base sequence to each of a plurality of areas, said areas being independent and formed on the surface of a substrate,

supplying a sample solution containing polynucleotides onto said substrate,

heating said sample solution up to a predetermined temperature and thereafter cooling the heated solution to thereby hybridize each of complementary polynucleotides separately to each of probes,

replacing said sample solution above the substrate with a solution containing no polynucleotide, and

heating the surface of the substrate at one area of said plurality of independent areas on the substrate up to a predetermined temperature, and thereby denaturing only a polynucleotide being hybridized complementarily to said probe immobilized on said area to extract said denatured polynucleotide.

2. A polynucleotide separation apparatus comprising:

a substrate having a plurality of independent areas, each of single stranded-oligonucleotide probes each having a specific base sequence being individually immobilized on each of said areas,

means for supplying a sample solution containing

polynucleotides onto said substrate,

means for replacing said sample solution above the substrate with a solution containing no polynucleotide,

temperature control means for heating said sample solution up to a predetermined temperature,

temperature control means for heating [the sample solution] the surface of the substrate at only one area of said plurality of independent areas on the substrate to a predetermined temperature, and

means for extracting said sample solution above the substrate.

3. A polynucleotide separation apparatus according to Claim 2, further comprising means for quantitatively detecting, separately on each of said areas, fluorescence emission intensity of a fluorescent dye with respect to each of said polynucleotides hybridized to said each probe of areas on the substrate, or intensity of autoemission fluorescence of said polynucleotides.

4. A polynucleotide separation apparatus according to Claim 3, wherein a light having wavelengths ranging from 280 nm to 650 nm is used as an exciting light for said observation of fluorescence.

5. A polynucleotide separation apparatus according to Claim 3, further comprising means for analyzing the temperatures of each of said areas separately based on changes of said quantitatively detected fluorescence emission intensity of the fluorescent dye attached to said individual polynucleotides hybridized separately to each

of said areas of the substrate or to said surface of the substrate, or based on changes of said quantitatively detected fluorescence autoemission intensity of said nucleotide sample.

6. A polynucleotide separation apparatus according to Claim 5, further comprising means for feedback-controlling the temperature of a specific area on the surface of the substrate separately, based on the analysis result concerning the temperature of each of said areas of the substrate obtained through said means for detecting fluorescence emission intensity emitted from the fluorescent dye.

7. A polynucleotide separation apparatus according to Claim 2, further comprising means for separately analyzing said temperature of each area of the substrate through a thermistor or a thermocouple.

8. A polynucleotide separation apparatus according to Claim 7, further comprising means for separately feedback-controlling the temperature of a specific area on the surface of the substrate, based upon said analysis result on the temperature of each of said areas of the substrate obtained through said analyzing means.

9. A polynucleotide separation apparatus according to Claim 2, further comprising thin film layers or particle layers having high photoabsorbing characteristics, each layer being formed separately at each of said areas on the substrate, and means for selectively irradiating a convergent light to a thin film layer or particle layer

at said specific area of the substrate, wherein said specific microarea is area-specifically heated through the photoabsorption of the light being selectively irradiated.

10. A polynucleotide separation apparatus according to Claim 9, wherein a light having a wavelength being not absorbed by any nucleotides is used as said convergent light for heating said microarea.

11. A polynucleotide separation apparatus according to Claim 9, wherein a substance absorbing lights each having a wavelength longer than 400 nm is applied, sprayed or vacuum-deposited on a substrate having a plurality of independent areas on its surface, and each of single stranded-oligonucleotide probes each having a specific base sequence being individually immobilized to each of said areas.

12. A polynucleotide separation apparatus according to Claim 9, wherein said exciting light used for the excitation in the florescent observation, said light for fluorescent observation and said convergent light for heating the microarea individually have a different wavelength from each other.

13. A polynucleotide separation apparatus according to Claim 2, further comprising a microsphere having an extremely higher photoabsorbing characteristics than the substrate, means for capturing said microsphere floating in the solution through a light radiation pressure generated by a convergent light having a

numerical aperture of equal to or more than 1.2, and means for moving arbitrarily said microsphere to the vicinity of said specific area on the substrate by said capturing means through the light radiation pressure and for heating said specific area on the substrate area-specifically.

14. A polynucleotide separation apparatus according to Claim 2, further comprising an array of heating element layers, each layer being attached to each of said areas of the substrate, and means for area-specifically heating said specific microarea by allowing one of said heating element layers to evolve heat.

15. A polynucleotide separation apparatus according to Claim 2, wherein said substrate is in the form of capillary having, on its inner surface, a plurality of independent split areas, each of different nucleotide probes being immobilized on each of said areas, and which apparatus comprising means for introducing a sample nucleotide solution into said capillary, temperature control means for hybridizing said probes to polynucleotide components in said sample solution, means for removing polynucleotides in the sample solution, which polynucleotides being not hybridized to said probes on the surface of the capillary, means for heating a specific area of said plurality of areas in the capillary to denature said polynucleotide component in the sample solution, said component having been hybridized to the probe at said area, and means for extracting said denatured polynucleotide component.

16. A polynucleotide separation apparatus according to Claim 15, comprising said means for introducing a sample nucleotide solution into said capillary, said temperature control means for hybridizing said probes to polynucleotide components in said sample solution, said means for removing polynucleotides in the sample solution, said polynucleotides being not hybridized to said probes on the surface of the capillary, means for placing a drop containing no nucleotide to come in contact with only a specific area of said plurality of areas in the capillary, means for heating said capillary to denature only said sample solution component being hybridized to the nucleotide probe at the area, said drop being in contact with said area, and means for extracting the drop containing said denatured nucleotide component.

17. A polynucleotide separation apparatus according to Claim 16, comprising said capillary having, on its inner surface, a plurality of cylindrically split independent areas, each of different nucleotide probes being immobilized to each of said areas, means for introducing a sample solution, a washing solution and air to said capillary, means for individually heating each of said areas of the capillary, means for heating said specific area of the capillary and denaturing said sample solution component being hybridized to the nucleotide probe at said area to extract said component.

18. A polynucleotide separation apparatus according to Claim 2, wherein said substrate has a metal

thin film layer on its surface, and said probes are separately immobilized through a metal oxide formed on the surface of said metal thin film layer and a crosslinking agent.

19. A polynucleotide separation apparatus according to Claim 18, wherein said metal oxide film layer absorbs a coherent light or a light having continuous wavelength, each having a wavelength of equal to or more than 350 nm and less than 633 nm.

20. A polynucleotide separation apparatus according to Claim 18, wherein said metal oxide film layer absorbs a coherent light or a light having continuous wavelength each having a wavelength of equal to or more than 633 nm and equal to or less than 1053 nm.

21. A polynucleotide separation apparatus according to Claim 18, further comprising a metal surface composed of an active residue A, a linker R and a metal Me having an oxidized surface and of the formula A-R-O-Me, wherein said polynucleotide probes are immobilized through said active residue A.

22. A polynucleotide separation apparatus according to Claim 21, wherein the active residue is introduced onto the oxide surface of the metal through a silane coupling reagent, and said polynucleotide probes are immobilized individually through said active residue.

23. A polynucleotide separation apparatus according to Claim 21, wherein said active residue A is a glycidoxy group, and wherein polynucleotide probes each

having an amino group are immobilized individually through said active residue A.

24. A polynucleotide separation apparatus according to Claim 18, wherein said metal oxide film layer has any oxide of a metal selected from the group consisting of Cr, Ti, V, Fe, Co, Ni, Mo and W.

25. A polynucleotide separation apparatus according to Claim 2, further comprising means for applying a DC field onto the surface of said substrate.

26. A polynucleotide separation apparatus according to Claim 25, further comprising means for applying said DC field while allowing the pH of the solution containing the sample to equal to or lower than 4 to attract nucleotide components alone to the surface of the substrate modified with nucleotide probes.

27. A polynucleotide separation apparatus according to Claim 2, further comprising means for applying an alternating field onto the surface of said substrate.

28. A polynucleotide separation apparatus according to Claim 2, further comprising a reservoir for retaining the sample solution, a substrate having a plurality of two-dimensionally split areas on its surface, each of said areas being modified with an oligonucleotide probe, means for applying an alternating or DC field individually to each of said areas of the substrate, means for allowing individually each of said areas of the substrate to evolve heat, and means for identifying an area

where said hybridized cell being present and for verifying the position of a cell dyed with a marker.

29. A polynucleotide separation apparatus according to Claim 2, wherein heating means capable of individually heating each of said areas to a temperature ranging from 60°C to 95°C is used.

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